

## **REMARKS**

### **I. Status of the Claims**

Claims 1, 4, 6, 8-27, 29-48 and 71 are pending and under consideration, claims 2, 3, 5, 7, 28 and 49-70 having been previously canceled. With this Amendment, claims 1, 10, 15, 27, 30, 35 and 71 are being amended, and claims 8, 9 and 29 are canceled herewith, without prejudice against their reintroduction into this or one or more timely filed continuation, divisional or continuation-in-part applications. Thus, after entry of this Amendment, claims 1, 4, 6, 10-27, 29-48 and 71 are pending and under consideration. The amendments of the claims and the various rejections raised in the Office Action are discussed in more detail, below.

### **II. Amendments**

Claims 1 and 27 are amended to specify that the plant cell having an intact cell wall is contained within a seed. Basis for this amendment can be found at least in original claim 29, as well as at page 10, paragraphs [0120] and [0123] of the published US Application 20060188992.

Claims 8, 9 and 29 are canceled herewith as redundant with amended claims 1 and 27.

Claims 10 and 15 amended for proper antecedent basis.

Claims 30, 35 and 71 are amended for proper dependency.

No new matter is added by way of these amendments.

### **III. Rejection under 35 U.S.C. §112, second paragraph**

Claims 8 and 9 were rejected under 35 U.S.C. §112, second paragraph as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention.

The cancelation of claims 8 and 9 obviates this rejection. Thus, Applicants respectfully request withdrawal of the rejection under 35 U.S.C. §112, second paragraph.

### **IV. Rejections under 35 U.S.C. §103(a)**

Claims 1, 4, 8-27, 29-48 and 71 were rejected under 35 U.S.C. §103(a) as allegedly obvious over U.S. Patent Publication 2005/0032212 (hereinafter "US '212").

Claims 1, 4, 8-27, 29-48 and 71 were rejected under 35 U.S.C. §103(a) as allegedly obvious over US '212 as applied above, in view of Dev (of record).

Claims 1, 4, 6, 8-27, 29-48 and 71 were rejected under 35 U.S.C. §103(a) as allegedly obvious over Rickwood (of record) in view of Dev.

Claims 1, 4, 6, 8-27, 29-48 and 71 were rejected under 35 U.S.C. §103(a) as allegedly obvious over Schmukler (of record) in view of Gutierrez-Armenta (of record) and Dev.

These rejections are respectfully traversed.

A. The Present Claims

As amended, the present claims refer to a method for improving the efficiency of transfer of a nucleic acid into a plant cell having an intact cell wall and contained in a seed, comprising the steps of a) holding a seed containing the cell under a pressure different from an atmospheric pressure wherein the pressure is reduced by about 0.096 MPa from the atmospheric pressure; b) placing the seed containing the cell and the nucleic acid under conditions to induce electroporation; and c) transferring the nucleic acid into the plant cell using electroporation (Claim 1); and a method for improving the efficiency of introducing a nucleic acid into a cell of a plant, wherein the cell has an intact cell wall, comprising the steps of a) holding a seed containing the cell under a pressure different from an atmospheric pressure wherein the pressure is reduced by about 0.096 MPa from the atmospheric pressure; b) placing the seed containing the cell and the nucleic acid under conditions to induce electroporation and introducing the nucleic acid into the cell using electroporation; and c) differentiating, growing, and/or multiplying the cell (Claim 27).

B. The Cited Art

US '212 discloses a method for permeabilising a viable cell having a cell wall, comprising (a) pressurising a fluid or gel in contact with a surface of the cell; and (b) depressurising the fluid or gel; to form at least one hole in a surface of the cells.

DEV disclose a method for producing genetically modified plants via electroporation in the absence of cell-wall degrading enzymes.

RICKWOOD discloses a method of transfecting cells involving the generation of bubbles of gas in a liquid medium which interact with the cell to be transformed and form a hole in the cell's surface.

SCHMUKLER discloses an apparatus and method for electroporation and electrofusion of cells, in particular myeloma and lymphoma cells, which are types of animal cells, as well as isolated nuclei (see Col . 3 of Schmukler).

GUTIERREZ-ARMENTA discloses the use of retinoblastoma protein to control growth of plant cells and/or plant viruses. Several methods of administering nucleotides to cells are disclosed, including electroporation of plant seed cells with DNA.

### C. Analysis

Applicants respectfully submit that the references, alone or in any combination, fail to teach or suggest all the limitations of the claims as presently amended. Specifically, the claims require transformation of a plant cell within a seed via electroporation and concurrent depressurization.

Furthermore, Office examination guidelines following the Court decision in *KSR International Co. v. Teleflex Inc.*, 82 USPQ2d 1385, 1385 (US 2007) indicate that an issue to consider in assessing obviousness is whether a combination of prior art elements yields "predictable results." See *Federal Register*, Vol. 72, No. 195, October 10, 2007. Applicants respectfully submit that the skilled artisan at the time of filing of the present application would not have predicted that the method, as presently claimed, would successfully transform a plant cell within a seed.

#### C1. Rejection of claims 1, 4, 8-27, 29-48 and 71 as allegedly obvious over US '212

Although US '212 discloses seed crops as candidate plants from which a viable cell having a cell wall may be permeabilized according to their method, US '212 fails to teach or disclose transformation of a plant cell within a seed. At most, paragraph [0059] of US '212 discloses suitable tissue types such as "meristem, disaggregated leaf cells, leaf discs, pollen, microspores (=immature pollen), cotyledon, callous tissue, somatic embryos, pre-embryonic masses, and all suspension culture tissue (=disaggregated cells comprising cell walls)" and paragraph [0226] of US '212 describes production of embryogenic rice callus from mature rice seeds by dehusking the seeds, sterilizing them and aseptically removing the embryos. The US '212 reference presents no working examples demonstrating successful introduction via electroporation of nucleic acids into plant cells in intact seeds using electroporation. Clearly this reference does not enable the skilled artisan to perform the presently claimed method. In fact, nowhere does the reference teach or even suggest the presently claimed method for improving the efficiency of transfer of a nucleic acid into a plant cell having an intact cell wall and contained in a seed, comprising the steps of a) holding a seed containing the cell under a pressure different from an atmospheric pressure wherein the pressure is reduced by about 0.096 MPa from the atmospheric pressure; b) placing the seed containing the cell and the nucleic acid under conditions to induce electroporation; and c) transferring the nucleic acid into the plant cell using electroporation.

Thus, because the references fails to teach or suggest all the elements required by the claims as presently amended, and because the skilled artisan at the time of filing of the present application would not have predicted that the method as presently claimed would successfully

transform a plant cell within a seed, Applicants respectfully request withdrawal of this rejection under 35 U.S.C. §103(a).

C2. Rejection of claims 1, 4, 8-27, 29-48 and 71 as allegedly obvious over US '212 in view of Dev

US '212 is described above. Dev describe the transformation of desiccated seed embryos in the absence of cell wall degrading enzymes, via electroporation. However, Dev fails to provide the missing teaching of transformation of a plant cell contained within a seed. As amended, the claims are not obvious over the combination of US '212 with Dev.

Thus, because the references fails to teach or suggest all the elements required by the claims as presently amended, and because the skilled artisan at the time of filing of the present application would not have predicted that the method as presently claimed would successfully transform a plant cell within a seed, Applicants respectfully request withdrawal of this rejection under 35 U.S.C. §103(a).

C3. Rejection of claims 1, 4, 6, 8-27, 29-48 and 71 as allegedly obvious over Rickwood in view of Dev

Rickwood discloses a method of introducing a nucleic acid, for example, into a cell wherein bubbles containing gas interact with the cell surface to form a hole in the surface of the cell, and the transfection can occur at a pressure below atmospheric pressure. Rickwood fails to teach or suggest the presently claimed method for improving the efficiency of transfer of a nucleic acid into a plant cell having an *intact cell wall and contained in a seed*, comprising the steps of a) holding a seed containing the cell under a pressure different from an atmospheric pressure wherein the pressure is reduced by about 0.096 MPa from the atmospheric pressure; b) placing the *seed containing the cell* and the nucleic acid under conditions to induce electroporation; and c) transferring the nucleic acid into the plant cell using electroporation.

While Rickwood generally suggests that the disclosed method can be applied to cells having a cell wall, such as plant cells, fungal cells and bacteria (page 9), it is clearly stated that “(i)n this latter embodiment, it is preferred that the method is carried out on a protoplast derived from the cell.” Clearly, such a method involving bubbles interacting with a cell surface to form holes in the cell surface would not work with *plant cells contained within a seed* and having an intact cell wall. Furthermore, as noted previously, the limits of Rickman’s disclosed range are from  $1 \times 10^4$  Pa to  $1 \times 10^5$  Pa, which encompasses a greater range of atmospheric pressures than the depressurized state required by the present claims in which the cell is held under a

pressure reduced by about 0.096 MPa from the atmospheric pressure (approximately  $4 \times 10^3$  Pa). The requirement in Applicants' claims for a pressure reduced by about 0.096 MPa from the atmospheric pressure" (approximately  $4 \times 10^3$  Pa) is neither taught nor suggested by Rickman. Thus, Rickman does not teach or suggest all the elements of the method as presently disclosed.

The secondary reference in this rejection, Dev, is discussed above, and adds nothing to cure the deficiencies of the Rickwood reference.

Thus, because the references fails to teach or suggest all the elements required by the claims as presently amended, and because the skilled artisan at the time of filing of the present application would not have predicted that the method as presently claimed would successfully transform a plant cell within a seed, Applicants respectfully request withdrawal of this rejection under 35 U.S.C. §103(a).

C4. Rejection of claims 1, 4, 6, 8-27, 29-48 and 71 as allegedly obvious over Schmukler in view of Gutierrez-Armenta and Dev

Schmukler teaches a method of electroporation in which cells are trapped into pores in a film with diameters smaller than the diameters of the cells, and an electric field is applied to cause electroporation. The Examiner points to column 3, lines 44-47, which teaches a pressure gradient across the film containing the pores. Schmukler also discloses an apparatus and method directed to electroporation and electrofusion of cells, in particular animal cells, as well as isolated nuclei (see Col . 3 of Schmukler). Schmukler fails to teach or suggest introduction of nucleic acids into plant cells, but rather, is directed to methods for fusing two types of cells, or fusing a cell with an isolated nucleus, rather than electroporation. As is true of US '212, Dev, and Rickwood, Schmucler also fails to teach or suggest the presently claimed method in which a plant cell having an intact cell wall and contained within a seed is transformed by electroporation and depressurization.

Gutierrez-Armenta teaches the electroporation of DNA into plant seed cells, but fails to teach or suggest transformation of a plant cell having an intact cell wall and contained within a seed. Thus, Gutierrez-Armenta, alone or in combination with Dev, discussed above, fail to supply the teaching missing from the Schmukler reference.

Accordingly, the references, alone or combined fail to teach all the elements, and would not give the skilled artisan at the time of filing of the present invention any reasonable expectation of success in transforming a plant cell having an intact cell wall within a seed by electroporation and depressurization.

**CONCLUSION**

Because none of the references, when considered either singly or in combination, teaches or suggests all the limitations of the present claims, nor is there any teaching or suggestion in the cited references to lead one skilled in the art to expect that the presently claimed method would successfully achieve transformation of a plant cell having an intact cell wall and contained within a seed, the standard for obviousness has not been met. Accordingly, Applicants respectfully request withdrawal of the rejection under 35 U.S.C. §103.

In view of the foregoing, claims 1, 4, 6, 10-27, 29-48 and 71 are believed to satisfy all of the criteria for patentability and are in condition for Allowance. An early indication of the same is therefore kindly requested.

No fees are believed to be due in connection with this Amendment. However, the Commissioner is authorized to charge any additional fees that may be required, or credit any overpayment, to King & Spalding LLP Deposit Account No. 50-4616.

If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 590-1932.

Respectfully submitted,  
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